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Selective recognition and electrochemical sensing of dopamine using a ferrocene-based heteroditopic receptor

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ABSTRACT

A redox-active ferrocene-based heteroditopic receptor bearing a boronic acid (as a catechol recognition site) and a benzo-18-crown-6-ether unit (as an ammonium ion recognition site) was synthesized. A 1:1 ditopic complex with dopamine was evidenced by mass spectrometry and NMR spectroscopy. Cyclic voltammetry measurements on the receptor in the presence of a series of organic guest species demonstrated the successful electrochemical sensing of dopamine through a distinct change in the ferrocene-centred redox-couple upon complex formation.

Dopamine (DA) is a neurotransmitter that plays an important role in the central nervous system, with DA levels significantly affecting brain function. Low levels of DA in vivo are associated with Parkinson's disease, while excessive amounts are associated with attention deficit hyperactivity disorder (ADHD) and motor dysfunction. Therefore, accurately determining DA levels is important in terms of the diagnosis of disease and monitoring its progression. Although techniques such as capillary electrophoresis[1] and high performance liquid chromatography (HPLC),[2] have been used to detect DA, electrochemical techniques[3] have an advantage as the measurement can be performed with high sensitivity, in real time and at low cost. However, since there are several compounds in vivo that have almost the same oxidation potential as DA,[4] it is very difficult to unambiguously identify and quantify DA levels in biological media through electrochemical means.[5,6] One potential solution to this issue is to develop redox-active receptors that signal the presence of the analyte at a separate oxidation potential, as has previously been demonstrated for other organic guest molecules.[7]

In this study, we report the synthesis of a ferrocene-containing receptor **1** designed to selectively bind and electrochemically sense DA as its hydrochloride salt. Compound **1** is a heteroditopic receptor[8-10] that simultaneously recognizes both the catechol moiety of the guest through its boronic acid[11-15] and the ammonium ion through its benzocrown ether (Figure 1).[16,17] We show that electrochemical detection of DA[18] is achieved by monitoring the change in the ferrocene-centred redox couple of **1** upon its binding in acetonitrile.

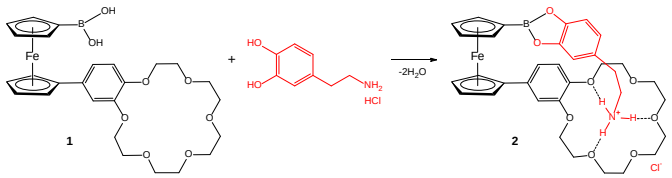
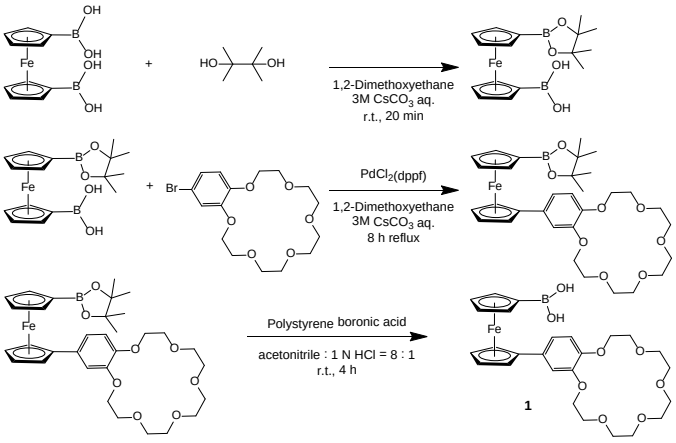


Figure 1. A ferrocene-based heteroditopic receptor **1** and its multipoint recognition of dopamine hydrochloride.



Scheme 1. Synthetic route to compound **1**.

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Synthesis of compound **1** was carried out after mono-protection of ferrocene diboronic acid using pinacol,[19] followed by the introduction of a benzo-18-crown-6-ether unit by Suzuki coupling.[20] The pinacol was then deprotected by polystyrene boronic acid[21] to give the compound **1** in overall yield of 28% (Scheme 1, see SI for experimental details).

A series of experiments were carried out to establish the binding properties of compound **1** with DA. The molecular ion peaks [**1**+H]$^+$ = 541.2, [**1**+Na]$^+$ = 563.2 and [**1**+K]$^+$ = 579.1 were clearly present in its ESI mass spectrum. When 1.0 molar equivalent of dopamine hydrochloride (DA·HCl) was added to a solution of **1** in CH_3CN containing 5.0% H_2O, these signals decreased at the expense of two new peaks of higher mass corresponding to the 1:1 complex with DA: [**1**-2H_2O+DA+H$^+$] = 658.2 and [**1**-2H_2O+DA·HCl+ H$^+$] = 694.3. This result clearly demonstrates the binding of compound **1** to DA, with the loss of two water molecules indicating the formation of the expected boronate ester adduct. In addition, the removal of metal ion adducts in the presence of DA is consistent with the formation of a ditopic complex **2** (Fig. 1), that also has a three-point H-bonding interaction between the protonated amine and the crown ether.

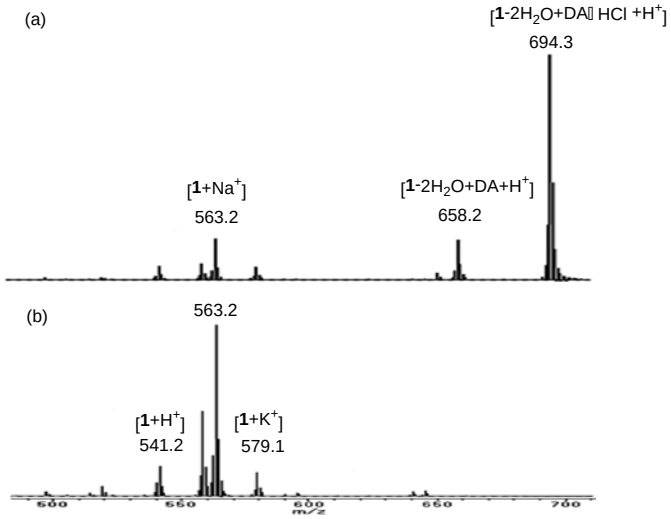

Figure 2. (a) The ESI mass spectrum of compound **1** (5.0% H_2O in CH_3CN) in (a) the presence and (b) the absence of 1.0 mol. equiv. of dopamine hydrochloride, DA·HCl.

The structure of complex **2** was calculated by DFT (B3LYP/6-31G(d,p)) using Gaussian,[22,23] which revealed that the receptor **1** was indeed able to accommodate DA·HCl through the formation of a ditopic complex, in which the catechol unit is recognized at the boronic acid site and the primary ammonium ion at the crown ether site (Figure 3).

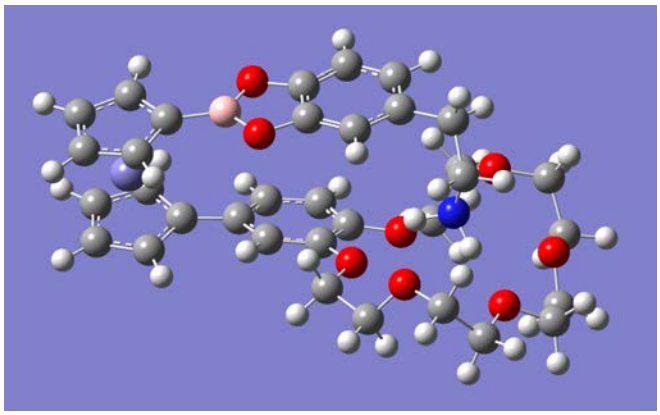

Figure 3. Optimized structure of complex **2** by DFT calculation.

A series of ^{1}H NMR experiments were then undertaken to probe the binding process further. DA·HCl (1.5 mol. equiv.) was added to 0.5 mM solutions of compound **1** in CD_3CN/H_2O with varying percentage compositions of water (see supplementary information). When the ratio of water was 1.5%,[24] four new signals at 4.3 ppm, 4.5 ppm, 4.7 ppm and 4.7 ppm (two overlapping), corresponding to the ferrocene cyclopentadienyl (Cp) protons (2 H each), were observed in the ^{1}H NMR spectrum at significantly different values to those for uncomplexed **1**. Furthermore, the characteristic multiplet at 3.5 ppm corresponding to the crown ether protons shifted slightly downfield to 3.7 ppm. In control experiments, no significant changes to the Cp proton resonances of **1** were observed when 1.5 equivalents of catechol (CA) and 2-phenylethylamine hydrochloride (2-PA·HCl) were added under the same conditions. This gives further evidence for the formation of a ditopic complex **2** between DA·HCl involving multiple point interactions that is facilitated by the ball-bearing effect of the ferrocene unit. Interestingly, under these conditions the exchange rate between unbound and bound forms of **1** was slower than the NMR time scale as signals originating from both unbound compound **1** and complex **2** were observed. Similar changes to the Cp and crown ether proton resonances were observed when the ratio of water was increased to 5.0% although the peaks for unbound **1** were significantly greater compared to the case for the 1.5% spectrum. Nevertheless, this indicates the ability of compound **1** to form a ditopic complex with DA·HCl even under more competitive solvent conditions.

In order to investigate the stoichiometry of the complex formed between compound **1** and DA·HCl, a Job plot was formed from the ^{1}H NMR spectra (3% H_2O in CD_3CN) by monitoring changes to the integration ratio of the newly appeared ferrocene Cp resonance at 4.5 ppm for the complex and the original ferrocene Cp resonance at 4.6 ppm for **1**. This indicated the expected 1:1 complex, albeit with a small amount of 1:2 (host:guest) complex also present (see supplementary information). Titration experiments were undertaken to assess the strength of the binding interaction between **1** and various guests by the curve fitting method.[25] The binding constant for the 1:1 complex with DA·HCl was determined to be K_a = 2,900 M^{-1} (1.5% H_2O in CD_3CN). As expected for a ditopic complex such as **2** displaying a positive cooperative binding effect, this value was considerably higher than those determined under the same conditions for catechol (CA) and 2-phenylethylamine hydrochloride (2-PA·HCl) which gave values of K_a = 0.8 M^{-1} and 1,100 M^{-1} respectively (see Figure 4 and supplementary information). It was also interesting to note that in contrast to the titration with DA·HCl, for 2-PA·HCl, no new peaks were observed upon complex formation, rather a gradual shift in the crown ether protons. This indicates fast guest exchange on the NMR timescale, which would be expected for a weaker complex that is not held so tightly by multiple point interactions found in **2**.

The ability of compound **1** to act as an electrochemical molecular sensor[7] for DA was then probed using cyclic voltammetry (CV). Compound **1** gave a characteristic redox couple at $E_{1/2}$ = 460 mV vs. Ag/AgCl in acetonitrile (1.5% H_2O), corresponding to the reversiobe oxidation of the ferrocene unit, where $E_{1/2}$ = (E_{pa} + E_{pc})/2. A series of compounds were then added to investigate the effect of complexation on this redox couple.

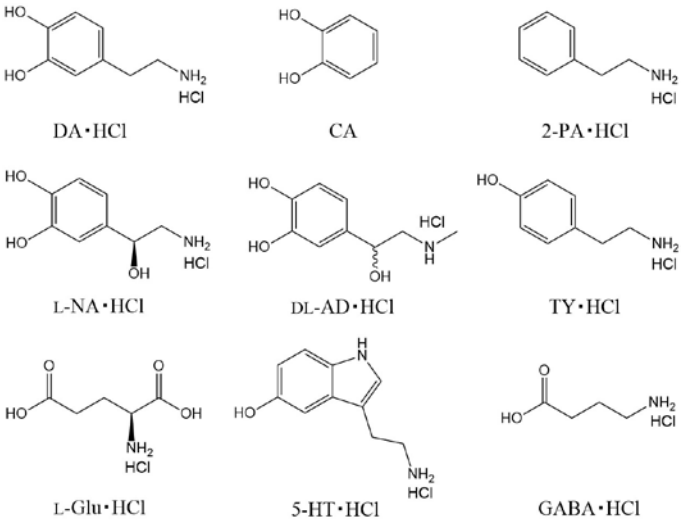

Figure 4. Guest molecules used in this study.

Figure 5 shows the change in the CV upon the addition of 1.5 molar equivalents of DA·HCl (dissolved in water) to compound **1** (acetonitrile, 0.5 mM). A significant change was observed, with the two main waves, best explained as corresponding to the oxidation and reduction of the 1:1 complex between **1** and DA, appearing at more positive potentials, giving a $E_{1/2}$ value of 510 mV and a $\Delta E_{1/2}$ value of +70 mV (see the supplementary information for more details). When the amount of DA·HCl was increased to 2.0 and 2.5 molar equivalents, no further significant changes were observed, which again supports the formation of the 1:1 ditopic complex.

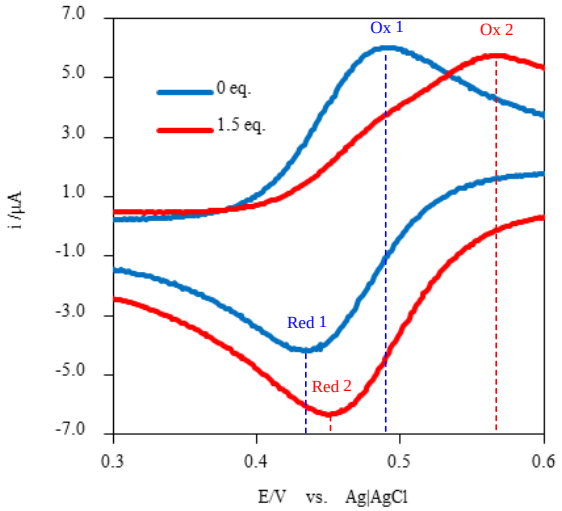

Figure 5. Cyclic voltammograms recorded for compound **1** (1.5% H_2O in CH_3CN) in the absence and in the presence of 1.5 mol. eq. of DA·HCl (sweep rate: 50 mV/s, electrode: GC, counter electrode: Pt).

In order to assess how distinct these changes were in terms of sensing and identifying DA electrochemically, a series of similar experiments was next undertaken with other organic molecules (Fig. 4, see also supplementary information). The addition of CA and 2-PA·HCl gave much lower $\Delta E_{1/2}$ values of +4 mV and +14 mV respectively. The presence of an ammonium ion bound in close proximity to the ferrocene unit would be expected to make the ferrocene unit harder to oxidise and shift the redox couple slightly to more positive potentials.[9] However the much larger value observed with DA·HCl gives further support for the formation of the ditopic complex **2**, in which the guest is strapped across the ferrocene unit, making a more marked effect on the electrochemical properties. Six other neurotransmitters were then tested in the same way including L-noradrenaline hydrochloride (L-NA·HCl), DL-adrenaline hydrochloride (DL-AD·HCl) and γ-aminobutyric acid hydrochloride (GABA·HCl) (Fig. 4). In all cases, less marked shifts were observed, with the greatest $\Delta E_{1/2}$ value of +30 mV observed for L-NA·HCl, presumably since this is the only guest species that can also form a multiple point ditopic complex,[26] having both a primary ammonium group and a catechol unit. Those compounds containing only a primary ammonium group and no catechol unit gave similar broadly similar $\Delta E_{1/2}$ values to PA·HCl, which was consistent with these guest species forming just a three-point H-bonding interaction with the benzocrown ether moiety.

In conclusion, we have succeeded in the synthesis of ferrocene compound **1** bearing both a boronic acid moiety and a benzocrown ether. It was found that this supramolecular receptor can form a 1:1 ditopic complex **2** with dopamine hydrochloride (DA·HCl), with binding able to be read out electrochemically, allowing it to function as an electrochemical sensor for this neurotransmitter.[27] The work demonstrates the ability to sense a redox-active target, not by monitoring the effect on its own redox properties but on those of a redox-active receptor to which it binds, thus allowing a different potential window to be monitored.

Acknowledgments

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24. In neat CD_3CN or in the presence of 1.0% H_2O, the binding constant between compound **1** and DA·HCl was too large to obtain an accurate value from ^1H NMR spectra (K_a> 10,000 M^{-1}). Therefore, to allow a comparison of binding constants, measurements were performed in 1.5% H_2O in CD_3CN.
25. The binding constants were determined by a non-linear curve fitting method using Origin (OriginLab co.).
26. The binding constants (K_a) between compound **1** and L-NA·HCl and DL-AD·HCl were 1,800 M^{-1} and 130 M^{-1} respectively, as determined from ^1H NMR titration experiments (1.5% H_2O in CD_3CN)
27. In this study, measurements were performed under standard CV conditions in organic solvents at millimolar concentrations. However, given a suitably strong binding interaction and more sensitive instrumentation, this approach could be used to detect lower concentrations of dopamine and in more competitive solvent conditions.